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Proposed Registration Document PRD2014-02

Phlebiopsis gigantea strain VRA 1992

(publié aussi en français)

13 January 2014

This document is published by the Health Canada Pest Management Regulatory Agency. For further information, please contact:

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Canada

ISSN: 1925-0878 (print)
1925-0886 (online)

Catalogue number: H113-23/2014-2E (print version)
H113-23/2014-2E-PDF (PDF version)

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Overview

Proposed Registration Decision for *Phlebiopsis gigantea* strain VRA 1992

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of Phlebiopsis gigantea strain VRA 1992 and Rotstop C, containing the technical grade active ingredient *Phlebiopsis gigantea* strain VRA 1992, to control root and butt rot, caused by *Heterobasidion irregularee*, on susceptible conifer species.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of *Phlebiopsis gigantea* strain VRA 1992 and Rotstop C.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment (for example, those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides and Pest Management portion of Health Canada's website at healthcanada.gc.ca/pmra.

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1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act.*

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (*a*) efficacy; (*b*) effect on host organisms in connection with which it is intended to be used; and (*c*) health, safety and environmental benefits and social and economic impact."

Before making a final registration decision on *Phlebiopsis gigantea* strain VRA 1992, the PMRA will consider all comments received from the public in response to this consultation document.[3] The PMRA will then publish a Registration Decision[4] on *Phlebiopsis gigantea* strain VRA 1992, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation of this consultation document.

What Is *Phlebiopsis gigantea* strain VRA 1992?

Phlebiopsis gigantea strain VRA 1992 is a naturally occurring saprophytic wood-rotting fungus that was isolated from a red pine stump in Harrington, Quebec. The fungus is a primary colonizer of wood, and requires high moisture content for its growth. It antagonizes the fungal pathogen *Heterobasidion irregulare* through competitive exclusion for space and nutrients. *Phlebiopsis gigantea* strain VRA 1992 is a microbial pest control agent (MPCA) in the technical product, Phlebiopsis gigantea strain VRA 1992, and the associated end-use product, Rotstop C. Rotstop C is a commercial fungicide proposed for control of root and butt rot (caused by *Heterobasidion irregulare*) in conifer trees.

Health Considerations

Can Approved Uses of *Phlebiopsis gigantea* strain VRA 1992 Affect Human Health?

***Phlebiopsis gigantea* strain VRA 1992 is unlikely to affect your health when Rotstop C is used according to the label directions.**

People could be exposed to *P. gigantea* strain VRA 1992 when handling and applying Rotstop C. When assessing health risks, several key factors are considered: the microorganism's biological properties (for example, production of toxic by-products); reports of any adverse incidents; its potential to cause disease or toxicity as determined in toxicological studies; and the level to which people may be exposed relative to exposures already encountered in nature to other isolates of this microorganism.

Toxicological studies in laboratory animals describe potential health effects from large doses in order to identify any potential pathogenicity, infectivity and toxicity concerns. When a comparative strain of *P. gigantea* (*P. gigantea* strain VRA 1835), was tested on laboratory animals, there were no signs that it caused any significant toxicity or disease. Furthermore *P. gigantea* strain VRA 1992 has an optimal growth temperature of 28°C and a maximum of 38°C. No adverse effects from *P. gigantea* strain VRA 1992 were reported in the published scientific literature.

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3 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

4 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

Residues in Water and Food

Dietary risks from food and water are not of concern.

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada sets science-based MRLs to ensure that the food Canadians eat is safe.

Phlebiopsis gigantea is ubiquitous in the forest environment and spores are commonly found in the air and on exposed surfaces. When *P. gigantea* strain VRA 1835 was administered orally to rats, no signs of toxicity or disease were observed, and no metabolites of toxicological significance have been shown to be produced by this strain of *P. gigantea*.

The end-use product has not been approved for food uses, therefore, as no residues of Rotstop C are expected on agricultural commodities, the establishment of an MRL is not required for *Phlebiopsis gigantea* strain VRA 1992. As well, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Consequently, dietary risks are minimal to non-existent.

Occupational Risks From Handling Rotstop C

Occupational risks are not of concern when Rotstop C is used according to label directions, which include protective measures.

Workers handling Rotstop C can come into direct contact with *P. gigantea* strain VRA 1992 on the skin, in the eyes or by inhalation. For this reason, the product label will specify that workers handling Rotstop C must wear waterproof gloves, long-sleeved shirts, long pants, a dust-mist filtering respirator/mask (NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator (with any N-95, P-95, R-95 or HE filter for biological products), and shoes plus socks. Respiratory protection for manual application by paint brush is not required.

This personal protective equipment is not required for operators of mechanical harvesters as they work in enclosed cabs.

As a product used in forestry, bystander exposure is expected to be much less than that of handlers and mixer/loaders and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When Rotstop C Is Introduced Into the Environment?

Environmental risks are not of concern.

Information available in the published literature on the environmental fate of *Phlebiopsis gigantea* strain VRA 1992 suggests that, as a saprophytic fungus, the organism will establish itself well in stumps and dead wood but rarely in standing trees. Survival of *P. gigantea* strain VRA 1992 in soil is limited and populations of *P. gigantea* strain VRA 1992 in soil should return to naturally occurring levels over time.

Waivers for toxicity testing on avian species, wild mammals, arthropods, non-arthropod invertebrates as well as for freshwater fish, aquatic arthropods, and aquatic plants were deemed acceptable to address the environmental toxicological requirements for these animals. The rationales were based on the ubiquitous nature of *P. gigantea* in the forest environment and that the level of *P. gigantea* in the terrestrial and aquatic environment will not significantly increase as a result of the use of Rotstop C as a stump treatment during forestry timber harvesting. The toxicity profile of *P. gigantea* strain VRA 1992 based on laboratory animal studies also demonstrated a lack of toxicity, and a review of published literature indicated no reports of adverse effects to these terrestrial organisms, as well as a lack of adverse effects to aquatic organisms from natural populations of *P. gigantea*.

In published literature, *P. gigantea* has demonstrated a limited ability to infect living trees and does not cause adverse effects to other forest-dwelling plants.

Published literature has shown that certain types of fungi and terrestrial insects dwelling within the stumps that will be treated with Rotstop C may be temporarily affected but microbial and insect populations are expected to gradually re-establish as the natural degradation processes take place in the treated stump.

Furthermore, forestry products containing other strains of *P. gigantea* have been used in Europe for decades with no reports of adverse effects to animals.

A toxicity study has also shown that *P. gigantea* is not toxic or pathogenic to honeybees.

Value Considerations

What Is the Value of Rotstop C?

Rotstop C is a microbial fungicide that provides control of root and butt rot, caused by *Heterobasidion irregulare*, on susceptible conifer species.

Root and butt rot is considered to be one of the most economically important diseases in European temperate coniferous forests and it has the potential to significantly impact managed red pine plantations in Canada. The registration of Rotstop C will provide forest managers and woodlot owners with a fungicide option to manage this disease, as no products are currently registered for this use in Canada.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures being proposed on the label of Rotstop C to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

In individuals exposed to large quantities of Rotstop C, respiratory and dermal sensitivity could possibly develop upon repeated exposure to the product since all microorganisms, including *P. gigantea* strain VRA 1992, contain substances that are potential sensitizers. Therefore, anyone handling or manually spraying Rotstop C must wear waterproof gloves, long-sleeved shirts, long pants, a dust-mist filtering respirator/mask (NIOSH approval number prefix TC-21C) or NIOSH-approved respirator (with any N-95, P-95, R-95 or HE filter for biological products), and shoes plus socks. Also, the signal words, "POTENTIAL SENSITIZER" are required on the principal display panel of Phlebiopsis gigantea strain VRA 1992 and Rotstop C and precautionary statements, "Avoid contact with eyes, skin and clothing," "Avoid breathing the dust or spray mist," and "May cause sensitization." are required on the secondary display panel of the label for Rotstop C.

Environment

The end-use product label will include environmental precaution statements that prevent the contamination of aquatic systems from the use of Rotstop C.

Next Steps

Before making a final registration decision on *Phlebiopsis gigantea* strain VRA 1992, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the Agency's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Phlebiopsis gigantea* strain VRA 1992 (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Phlebiopsis gigantea strain VRA 1992

1.0 The Active Substance, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Phlebiopsis gigantea* strain VRA 1992
Function	To control butt and root rot caused by *Heterobasidion irregulare* in conifer species.
Binomial name	*Phlebiopsis gigantea* strain VRA 1992
Taxonomic designation	
Kingdom	Fungi
Phylum	Basidiomycota
Class	Basidiomycetes
Order	Polyporales
Family	Phanerochaetaceae
Genus	*Phlebiopsis*
Species	*gigantea*
Strain	VRA 1992
Patent Status information	The production process for Rotstop is protected by the following patent: CA2581363. Priority date 2004-09-28. Application number: 20041253 Country: Finland.
Minimum purity of active	Phlebiopsis gigantea strain VRA 1992 (technical grade of the active ingredient): 94% at $>1.0 \times 10^7$ CFU/g Rotstop C (EP): 10% at $>1.0 \times 10^6$ CFU/g
Identity of relevant impurities of toxicological, environmental and/or significance.	The technical grade of the active ingredient does not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards. *Phlebiopsis gigantea* strain VRA 1992 is not known to produce any toxic secondary metabolites (see Section 3.0).

1.2 Physical and Chemical Properties of the Active Ingredients and End-Use Product

Technical Product – Phlebiopsis gigantea strain VRA 1992

Properties	Phlebiopsis gigantea strain VRA 1992
Physical state	Fungal cell mass and spent media
Colour	Brown
Odour	Fungus smell
pH (1% w/v)	6.0
Guarantee	$\geq 1.0 \times 10^7$ CFU/g ; 94.0% w/w
Corrosion Character	None
Viscosity	Not applicable

End-Use Product – Rotstop C

Properties	Rotstop C
Physical state	Fine powder
Colour	Cream opaque
Odour	Weak fungus smell
pH (1% w/v)	6.3
Guarantee	$1.0 \times 10^6 - 1.0 \times 10^7$ CFU/g; 10.2 % w/w
Corrosion Character	None
Viscosity	Not applicable

1.3 Directions for Use

Rotstop C is to be applied to the surface of freshly-cut conifer stumps, within three hours after tree felling, at a rate of at least 1 g/L water/m^2 of stump area. Application can be made with a brush, a hand sprayer, a backpack sprayer or using an application device mounted on a forest harvester.

1.4 Mode of Action

The mode of action of *Phlebiopsis gigantea* against *Heterobasidion irregulare* is direct competition for the wood substrate, as both fungi share the same ecological niche. When applied to freshly-cut stumps, *P. gigantea* will out compete *H. irregulare* for space and nutrients, thereby eliminating its main infection route.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

Appropriate methodologies for detection, isolation and enumeration of the active ingredient, *P. gigantea* strain VRA 1992, were submitted by the applicant. The MPCA has been fully characterized with respect to its origin of strain, natural occurrence and biological properties. *Phlebiopsis gigantea* strain VRA 1992 can be identified to the species level using a combination of colony morphologies on agar media and to the strain level using the latest DNA-based methodologies.

2.2 Method for Establishment of Purity of Seed Stock

A stock culture of *P. gigantea strain* VRA 1992 is maintained in the American Type Culture Collection and German Collection of Microorganisms and Cell Cultures. Stock cultures are kept frozen at -80°C.

Practices for ensuring the purity of the seed stock were adequately described in the method of manufacture and quality assurance program.

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

The potency (CFU/g) of the technical grade active ingredient and the end-use product will be determined by plate counting on agar media using the most probable number (MPN) method for enumeration.

2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

As the end-use product has not been approved for food uses, no methods to determine and quantify the MPCA and relevant metabolites are required.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures that will be used to limit contaminating microorganisms during manufacture of technical product and Rotstop C are acceptable.

During manufacturing, several approaches will be used to monitor microbial contamination in the technical and associated end-use product. These approaches will include frequent purity checks on agar media, sterilization of all equipment and media, and sanitization of recovery equipment.

The absence of human pathogens and below-threshold levels of contaminants was demonstrated in representative batches using pathogen-specific growth media. Microbe-specific screening methods for enteric bacteria/total coliforms, yeasts/moulds, *Salmonella* spp., *Shigella* spp. and *Staphyloccocus aureus* are adequate for detecting and enumerating microbial contaminants of concern. Release standards for microbial contaminants in the production batches comply with those permitted by the PMRA and are adequate to ensure that the end-use products do not contain unacceptable levels of human and animal disease-causing microorganisms.

No known toxic metabolites or hazardous substances are present in Rotstop C.

2.6 Methods to Determine Storage Stability, Shelf-life of the Microorganism

Results from storage stability testing of Rotstop C showed that these products are stable for one year when stored refrigerated below 8°C, or 5 months at room temperature (22°C). No storage stability data are required for the technical grade of the active ingredient since it is not stored as a separate product.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

The PMRA conducted a detailed review of the toxicological database submitted for *P. gigantea* strain VRA 1992. The database is complete, however it should be noted that the laboratory studies were conducted using the European authorized end-use product (Rotstop) containing *P. gigantea* strain VRA 1835. Given the similarities between North American and European populations of *P. gigantea* as regards their ecological and physiological properties and partly also their genetic make-up, the results of the mammalian testing are considered appropriate to allow for a decision on registration in Canada for products containing *P. gigantea* strain VRA 1992. The results of laboratory animal (in vivo) toxicity studies (acute oral toxicity/pathogenicity, acute pulmonary toxicity/pathogenicity, acute intraperitoneal infectivity, acute dermal toxicity, dermal sensitization and irritation, and eye irritation) currently required for health hazard assessment purposes were considered to be acceptable.

In an acute oral toxicity/pathogenicity study, no significant toxicity, no infectivity and no pathogenicity were observed in Sprague-Dawley rats following oral gavage with 4.26×10^7 CFU/kg bw of *P. gigantea* strain VRA 1835. There was a rapid loss of viability observed and the test substance was never detected in blood or organs of the rats. Based on the results of this study, *P. gigantea* strain VRA 1835 is of low toxicity and is not pathogenic/infective in the rat when challenged via the oral route.

In a pulmonary toxicity/pathogenicity study, no significant toxicity was observed in Sprague-Dawley rats following intratracheal treatment with Rotstop at a dose of 1.12×10^6 CFU of *P. gigantea* strain VRA 1835/kg bw. Within 24 hours of dosing five rats died in treated groups (viable and the autocalved test substance) and there were a number of clinical signs in treated rats (including but not limited to, lethargy, piloerection, decreased and increased respiratory rate,

unsteadiness and red staining). These deaths and the clinical signs were attributed to the effects of the anesthesia and trauma of the dosing procedure. There was a rapid loss of viability following intratracheal dosing and the test substance was only detected in the lungs of rats that died as a result of dosing procedure, not in blood or organs of any other treated rats. No adverse effects attributed to the test substance were observed. Based on the results of this study *P. gigantea* strain VRA 1835 is of low toxicity and is not pathogenic/infective in the rat when challenged via the pulmonary route.

In an acute intraperitoneal infectivity study, no mortalities and no treatment related clinical signs, or changes in body weights and organs were observed in Sprague-Dawley rats following injection with a dose of 1.82×10^5 CFU/animal of *P. gigantea* strain VRA 1835. The test substance was never detected in blood or organs of the rats. Although there was a rapid loss of viability of *P. gigantea* strain VRA 1835 following dosing, there was evidence of some toxicity as revealed by white nodules on the organs of some animals treated with the viable test substance. These nodules were not present in any animals in the autoclaved test substance or untreated groups. Based on the results, there was no evidence of pathogenicity observed in rats following intravenous injection with *P. gigantea* strain VRA 1835 at 1.82×10^5 CFU/rat.

In an acute dermal toxicity study, New Zealand rabbits were treated with Rotstop containing *P. gigantea* strain VRA 1835 at a dose of 2000 mg/kg body weight over 10% of the total body surface for 24 hours. All animals were lethargic up to 48 hours following dosing. Dermal irritation (maximum irritation score; MIS of 0.3/4 at 72h) was observed in four animals, and was completely resolved by Day 10. No dermal irritation was observed in the remaining six animals during the study and no mortalities or other signs of toxicity were observed.

In a skin sensitization study, guinea pigs were treated with 0.5 mL of Rotstop (1.07×10^7 CFU *P. gigantea* strain VRA 1835/g), in sterile physiological saline. Rotstop was not a dermal sensitizer.

In an eye irritation study, a single instillation of 40 mg of Rotstop into the eye of the rabbit elicited corneal opacification and conjunctival irritation. However, because the Rotstop formulation contained a red dye the results of the conjunctival irritation were inconclusive. The reactions had resolved by Day 7 or Day 14. Based on the maximum average score (MAS) of 8.5 (Day 2 time point) Rotstop is minimally/slightly irritating to the eye.

Higher tier subchronic and chronic toxicity studies were not required because of the low acute toxicity of the MPCA, and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary toxicity/infectivity tests.

Phlebiopsis gigantea is a saprophytic, wood-rotting basidiomycete fungus and is not listed in the scientific literature as a toxic organism. It is not expected to grow at mammalian body temperatures. *Phlebiopsis gigantea* is a non-pathogenic fungus, it is not thermophilic and has an optimal growth temperature of 28°C and a maximum of 38°C, and it has not been shown to be capable of colonizing or invading humans or animals, as confirmed by animal testing. Further animal testing with Rotstop, showed no irritation of skin, eyes or respiratory organs, and there are no records of adverse effects on operators and personnel handling *P. gigantea*-based products, in other words, no irritation or allergenic reactions that can be attributed to the MPCA in the products.

As many fungal species produce toxic secondary metabolites, the potential for *P. gigantea* strain VRA 1992 to produce mammalian toxins was considered in the assessment of risk.

Lup-19(22)-ene and Lupa-15,19(22)-diene are typical secondary fungal metabolites, and substances like this have been found in almost all wood-inhabiting fungi, including *P. gigantea*. Lup-19(22)-ene and Lupa-15,19(22)-diene belong to a class of substances which are widely distributed in nature, for example, in the bark of trees, in leaves and stems of annual plants, or in seeds. The toxicity of these compounds is lower than many other secondary fungal metabolites. Based on the toxicity, these compounds are not expected to cause harm as a result of the proposed stump treatment with Rotstop C. In the scientific literature there have been no other records of metabolites produced by *P. gigantea* that would be of concern for human health and/or the environment. Furthermore *P. gigantea* does not depend on the production of toxins for its ability to combat *H. irregulare*, but acts through competition for the wood resource.

Molecular and morphological analysis of the taxonomic position of *P. gigantea* although on-going, suggests a close relationship with the genera *Phanerochaete* and *Phlebia*. A database search of DIALINDEX was conducted using search terms "ALL SCIENCE". There were no reports of adverse effects to human or animal health associated with *Phanerochaete, Peniophora, Phlebia, and Pheniophora* and under the general family of *Phanerochaetaceae*. Of the genera grouped under *Phanerochaetaceae* there were no reports of deleterious impacts on human (or other animal) health.

Within the available scientific literature, there are no reports that suggest *P. gigantea* has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following oral, intraperitoneal and pulmonary routes of exposure, there is a rapid loss of viability by *P. gigantea*. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *P. gigantea* strain VRA 1992.

3.2 Occupational / Bystander Exposure and Risk Assessment

3.2.1 Occupational

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with primary exposure routes being dermal and/or inhalation. Since unbroken skin is a natural barrier to microbial invasion of the

human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Phlebiopsis gigantea* strain VRA 1992 has not been identified as a wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Furthermore, dermal toxicity studies in animals demonstrated no signs of systemic toxicity to *P. gigantea* strain VRA 1835.

The toxicity testing with *P. gigantea* strain VRA 1835 showed no significant signs of toxicity or infectivity via the oral, dermal, pulmonary, intraperitoneal routes of exposure. Although dermal toxicity or toxicity from inhalation exposure is considered minimal from the proposed EP use, the PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing. Risk mitigation measures, such as personal protective equipment, including waterproof gloves, long-sleeved shirts, long pants, a dust-mist filtering respirator/mask (NIOSH approval number prefix TC-21C) or NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter for biological products), and shoes plus socks are required to minimize exposure and protect mixer/loaders, applicators and handlers that are likely to be primarily exposed. Also, the signal words, "POTENTIAL SENSITIZER" are required on the principal display panel of *Phlebiopsis gigantea* strain VRA 1992 and Rotstop C and precautionary statements, Avoid contact with eyes, skin and clothing." "Avoid breathing the dust or spray mist." and "May cause sensitization." are required on the secondary display panel of the label for Rotstop C. Respiratory protection for manual application by paint brush is not required. The requirement for personal protective equipment is reduced for operators of the mechanized tree harvesters, such workers are required to wear long sleeved shirts and long pants, plus shoes and socks as a minimum.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of Rotstop C, and no significant occupational risks are anticipated for this product.

3.2.2 Bystander

Overall, the PMRA does not expect that bystander exposure will pose an undue risk on the basis of the low toxicity/pathogenicity profile for the MPCA and the assumption that precautionary label statements will be followed by commercial applicators in the use of Rotstop C.

The label does not allow applications to turf, residential or recreational areas; therefore, non-occupational dermal exposure and risk to adults, infants and children are low. Because the use sites are forestry, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

3.3 Incident Reports Related to Human and Animal Health

Since 26 April 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website www.healthcanada.gc.ca/pesticideincident. Incidents from Canada and the Unites States were searched and reviewed for *P. gigantea* strain VRA 1992.

As of 26 June 2013, there have been no incidents related to health or the environment reported to the PMRA, nor summarized by the USEPA or the California Department of Pesticide Regulation (CalDPR), for products containing *P. gigantea* strain VRA 1992.

In the decades of use in the laboratory and in the field, the Finnish Forest Research Institute has reported no allergic reactions or other kinds of sensitization in forestry workers, caused by European authorized strains of *P. gigantea*.

3.4 Dietary Exposure and Risk Assessment

3.4.1 Food

There are no proposed uses on food or feed crops and dietary exposure from food is not expected from the proposed use of Rotstop C.

Furthermore, higher tier subchronic and chronic dietary exposure studies were not required because of the low toxicity of the MPCA and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary and subcutaneous injection toxicity/infectivity studies. Therefore, there are no concerns for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

3.4.2 Drinking Water

The likelihood of *P. gigantea* strain VRA 1992 entering neighbouring aquatic environments or surface water run-off from the proposed use of Rotstop C as a stump treatment is considered very low. Although heavy rainfall might carry *P. gigantea* strain VRA 1992 into aquatic environments (for example, run-off from treated stumps), the MPCA is not expected to proliferate in aquatic habitats.

No risks are expected from exposure to this microorganism via drinking water because exposure will be minimal and there were no harmful effects observed in animals that were exposed orally in Tier I acute oral toxicity and infectivity testing. The RotStop C label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wastes. Furthermore, municipal treatment of drinking water will likely remove the transfer of residues to drinking water. Therefore, potential exposure to residues of *P. gigantea* in drinking water is negligible.

3.4.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

As there are no proposed uses to food or feed crops and given that the potential exposure to residues of *P. gigantea* strain VRA 1992 in drinking water is negligible, there is no concern for risks posed by dietary exposure of the general population, including infants and children, or animals to *P. gigantea* strain VRA 1992.

3.5 Maximum Residue Limits

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada sets science-based MRLs to ensure the food Canadians eat is safe.

Phlebiopsis gigantea are ubiquitous organisms found in most forest environments. Residues of *P. gigantea* strain VRA 1992 are not expected on agricultural commodities based on the stump treatment use. In addition, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Therefore, the PMRA has determined that an MRL does not need to be established for *P. gigantea* strain VRA 1992.

3.6 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information in the PMRA's files, there is reasonable certainty that no harm will result from aggregate exposure of residues of *P.gigantea* strain VRA 1992 to the general Canadian population, including infants and children, when the microbial pest control product is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Dermal and inhalation exposure to the general public will be very low since the product is to be used in forestry sites and is not allowed for use on turf, residential or recreational areas. Furthermore, few adverse effects from exposure to *P. gigantea* encountered in the environment have been reported. Even if there is an increase in exposure to this microorganism from the use of Rotstop C, there should not be any increase in potential human health risk.

3.7 Cumulative Effects

The PMRA has considered available information on the cumulative effects of residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Besides naturally occurring strains of *P. gigantea* in the environment, the PMRA is not aware of any other microorganisms, or other substances that share a common mechanism of toxicity with *P. gigantea* strain VRA 1992. No cumulative effects are anticipated if the residues of *P. gigantea* strain VRA 1992 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

No studies were submitted to address the environmental fate and behaviour of *P. gigantea* strain VRA 1992. Environmental fate data (Tier II/III) are not normally required at Tier I, and are only triggered if significant toxicological effects in non-target organisms are noted in Tier I testing.

Although no environmental fate studies were included, a literature search discussing the occurrence and fate of *P. gigantea* in the forest environment was submitted.

Phlebiopsis gigantea is a basidiomycete fungus that is found in conifer forests throughout the temperate Northern Hemisphere as well as in southern Europe, East Africa, Central America, Australia and New Zealand. *Phlebiopsis gigantea* is one of the first fungi to colonize recently cut stumps and timber and it is a relatively quick-growing saprophytic species once inhabiting moribund wood (for example, fallen branches/trunks, cut stumps, log piles, timber lying on the ground). As a natural component of forest ecosystems its spores are abundant in the air and on most exposed surfaces, particularly in the warm season. As a true saprophyte, *P. gigantea* rarely establishes itself in standing trees. In natural settings, basidiospsores (sexual) of *P. gigantea* are much more common than oidia (asexual spores). Basidiospores can survive in soil for several months under ideal conditions, but viability decreases steadily over time. In contrast, oidia are highly sensitive to environmental conditions and do not survive under unfavourable conditions.

Rotstop is the tradename of a series of *P. gigantea*-based products, containing European isolates of *P. gigantea* that have been used in forestry in Europe for decades. As the MPCA is present in the end-use product as oidia, the proposed use of Rotstop C will result in oidia levels that are much lower than the levels of naturally occurring basidiospores.

Published literature includes several studies which have examined the persistence of this fungus following stump treatment. Results vary but in general, *P. gigantea* can be recovered up to six years after treatment depending on a variety of factors, such as the tree species, the natural presence of other mycoflora and the rate of fungal succession, and the suitability of the environmental conditions. Although the MPCA can persist in the forest environment for several years, a published field study showed that new stumps created in forests that had been previously treated with Rotstop did not show an increase in occurrence of the MPCA.

Dispersal of *P. gigantea* within the forest has also been studied. While basidiospores of *P. gigantea* are highly mobile in air, oidia, which are in the end-use product, demonstrate more localized dispersal through air or via insect vectors to adjacent stumps only. However, one field study showed that the Rotstop isolate could be recovered six years after treatment from spruce stumps which were not themselves treated but which stood within the treated plot. The study remarked that untreated pine stumps were not colonized by the Rotstop isolate, suggesting that successful dispersal of the MPCA is dependent on a number of factors; tree species, the rate of fungal succession, temperature, and moisture are all likely to play a role.

In another study with an isolate from Quebec (*P. gigantea* strain P104), it was observed that artificial introduction of the biocontrol agent allowed for a faster and more uniform colonization of red pine stumps compared to natural infections of the fungus which took longer to establish itself deep into the stump.

Overall, as the end-use product contains oidia of the MPCA, it can be determined that the proposed use of Rotstop C will result in levels of oidia of *P. gigantea* that are much lower than the levels of naturally occurring basidiospores of this organism. Based on the information available on the fate of *P. gigantea* in the environment, the proposed application of Rotstop C to stumps in forests during thinning practices is not expected to result in a sustained increase of populations of the MPCA beyond those of naturally occurring forest dwelling *P. gigantea* species found in the environment.

4.2 Effects on Non-Target Species

The PMRA has a four-level tiered approach to environmental testing of microbial pesticides. Tier I studies consist of acute studies on up to seven broad taxonomic groups of non-target organisms exposed to a maximum hazard or Maximum Challenge Concentration (MCC) of the MPCA. The MCC is generally derived from the amount of the MPCA or its toxin expected to be available following application at the maximum recommended label rate multiplied by uncertainty factors. Tier II studies consist of environmental fate (persistence and dispersal) studies as well as additional acute toxicity testing of MPCAs. Tier III studies consist of chronic toxicity studies, in other words, life cycle studies, as well as definitive toxicity testing (for example, LC_{50}, LD_{50}). Tier IV studies consist of experimental field studies on toxicity and fate, and are required to determine whether adverse effects are realized under actual use conditions.

The type of environmental risk assessment conducted on MPCAs varies depending on the tier level that was triggered during testing. For many MPCAs, Tier I studies are sufficient to conduct environmental risk assessments. Tier I studies are designed to represent "conservative" scenarios

where the exposure conditions greatly exceed the expected environmental concentrations. The absence of adverse effects in Tier I studies are interpreted as minimal risk to the group of non-target organisms. However, higher tiered studies will be triggered if significant adverse effects on non-target organisms are identified in Tier I studies. These studies provide additional information that allows the PMRA to refine the environmental risk assessments. In the absence of adequate environmental fate and/or field studies, a screening level risk assessment can be performed to determine if the MPCA is likely to pose a risk to a group of non-target organisms. The screening level risk assessment uses simple methods, conservative exposure scenarios (for example, direct application at a maximum application rate) and sensitive toxicity endpoints. A risk quotient (RQ) is calculated by dividing the exposure estimate by an appropriate toxicity value (RQ = exposure/toxicity), and the risk quotient is then compared to the level of concern (LOC).

If the screening level risk quotient is below the level of concern, the risk is considered negligible and no further risk characterization is necessary. If the screening level risk quotient is equal to or greater than the level of concern, then a refined risk assessment is performed to further characterize the risk. A refined assessment takes into consideration more realistic exposure scenarios (environmental fate and/or field testing results). Refinements to the risk assessment may continue until the risk is adequately characterized or no further refinements are possible.

4.2.1 Effects on Terrestrial Organisms

A waiver request was submitted to address the potential for adverse effects from *P. gigantea* on avian species, wild mammals, non-arthropod invertebrates, and soil-microorganisms in lieu of non-target organism testing. Two reviews of published studies examining the pathogenicity of *P. gigantea* to trees were submitted to specifically support the toxicity to non-target trees, as well as a broader literature review of published studies relating to the use of *P. gigantea* in forestry. Also submitted was a review of a published study addressing the toxicity of Rotstop C to ground vegetation. Furthermore, a comprehensive review of published literature was submitted to address the impact of Rotstop C on stump mycoflora and terrestrial arthropods. A dietary/contact toxicity study on honeybees was also submitted.

Requests to waive toxicity testing of avian species, wild mammal, and non-arthropod invertebrates and soil microorganisms were accepted based on the following rationale. The MPCA, *P. gigantea* strain VRA 1992, is a naturally-occurring wood-decay fungus isolated from a red pine stump in Quebec. The end-use product is applied as a single application which is sprayed (or brushed) on as a thin layer (1 g/L water/m^2; 1 mm thickness) directly on the stump surface after felling. One application provides protection for several years. Based on this use pattern, direct exposure from *P. gigantea* strain VRA 1992 to non-target terrestrial organisms, including birds, wild mammals, earthworms, and soil microorganisms, is minimal. For soil microorganisms in particular, natural background levels of the MPCA are not expected to be considerably increased in the soil from this use pattern, and therefore environmentally or economically important microbial species are not expected to be affected, nor are any microbiologically-mediated biogeochemical processes expected to be affected. Furthermore, *P. gigantea*-based products have been used in forests for decades in Europe with no reports of adverse effects to non-target organisms, thereby establishing a history of use of *P. gigantea*.

An extensive literature search was also conducted to determine if there have been any reports in published literature of adverse effects from *P. gigantea* to avian species, wild mammals, earthworms or soil microorganisms. There have been no reports of toxicity or adverse effects from natural populations of *P. gigantea* to any of these non-target organisms, nor does the MPCA appear on any authoritative list of pathogens of any of these non-target organisms. The MPCA also does not thrive at mammalian or avian body temperatures and toxicity studies submitted to address the Human Health and Safety Testing requirements (DACO M4) further support a lack of toxicity and infectivity for *P. gigantea* to mammals.

The potential for adverse effects to non-target trees and plants in the vicinity of stumps treated with Rotstop C was addressed with a literature review. Overall, *P. gigantea* shows a lack of infectivity and a lack of persistence in living trees. However, in artificially created wounds the MPCA did show an ability to colonize living tissue; this observation is not unexpected as the ability to degrade tissue is considered a necessary requirement for any successful microbial biocontrol agent. None of the conditions under which infectivity was observed (in other words, high inoculum concentrations; bypassing the protective bark barrier with direct exposure deep into sapwood) are expected to arise from the use of Rotstop C on stumps at low inoculum levels. Published literature also reported no adverse effects from *P. gigantea* to ground vegetation in close proximity to the stumps following treatments with the MPCA. Based on the use pattern where Rotstop C is applied at low levels directly to the stump surface, exposure to non-target trees within the use site of the forest is expected to be minimal. Moreover, products containing *P. gigantea* have been used in Europe for stump treatment for decades with no reported adverse effects to living trees growing within the forest stands.

Detrimental effects on genetic variation of *P. gigantea* populations from the long-term use of Rotstop C is also not expected to pose a concern since the organism is heterothallic in its natural habitat, thereby requiring two genetically distinct, and compatible, mycelia for successful conjugation. In fact, products containing *P. gigantea* have been used in Europe since the 1970s and a study has shown that the level of genetic variation in *P. gigantea* within Europe remains high. Consequently, a significant level of genetic variation within the species is expected. Furthermore, as the product contains a Canadian isolate, potential concerns associated with introducing a non-indigenous isolate are alleviated. Rotstop C is also applied as a single application in minimal amounts (1 g/L water/m^2) which reduces the likelihood of detrimental effects on genetic variability compared to far more intense use patterns. Should the need arise manufacturers could periodically substitute the isolates in the biological product to further alleviate any concerns.

A published literature search was submitted to address the potential for adverse effects on terrestrial arthropods as well as on the mycoflora within the stump being treated with the European product, Rotstop.

Based on field and laboratory studies in published literature it appears that some insect species live in close association with *P. gigantea* without adverse effects (for example, red turpentine beetle; *Hylobius abietis*) while other species are negatively affected by the fungus (for example, the large pine weevil). The fungus was considered to have exerted its antagonistic effects by consuming the nutrient-rich cambium layer of the stump that would have been utilized by the developing larvae and adult insects.

Based on Canadian and European field studies investigating effects on mycoflora, the extent of the impact of *P. gigantea* treatment on stump mycoflora varies according to the duration of the study, the host species studied, and various environmental factors. Based on the mode of action of the product, effects on fungal species composition can be expected as the MPCA outcompetes certain fungi for substrate and living space. As Rotstop C is effective for several years, this prolonged effect is directly attributed to the temporal shift in mycoflora within the stump. However, it is understood that a temporal effect in fungal composition in stumps on its own does not necessarily mean a loss of diversity in the forest ecosystem on the whole.

Overall, while temporal and localized changes in insect populations and in the microbial community can be expected from the stump treatment with *P. gigantea*, these non-target populations are expected to gradually re-establish as the natural decay processes take place in the treated stump. Furthermore, forestry products containing *P. gigantea* have been used in Europe for decades with no evidence of an increase in fungal disease or insects pest outbreaks in forestry that can be attributed to the use of Rotstop. Also as the MPCA is native to Canadian forests, it is not expected that artificial introduction of a native species, particularly from a single application at such low levels, would greatly alter the existing fungal and insect population.

The potential for adverse effects to honeybees (*Apis mellifera*) was specifically assessed in a toxicity study with Rotstop, a product containing *P. gigantea* strain VRA 1835, a Finnish isolate. A range-finding study was conducted concurrently to a limit test. In the range-finding study, groups of honeybees were exposed to a single dose of Rotstop (4×10^6 CFU/g) at rates ranging from 0.01–100 µg/bee either in the diet or on the dorsal surface or at a single dose of 100 µg/bee in the diet or on the dorsal surface for the limit test. Based on the 48-hour (oral and contact) LD_{50} value for honeybees of >100 µg product/bee, Rotstop was considered to be non-toxic via oral and contact administration.

Based on all the available information on *P. gigantea* there is reasonable certainty that the proposed use of Rotstop C in forestry will not cause harm to birds, wild mammals, terrestrial arthropods, terrestrial non-arthropod invertebrates, terrestrial plants (including non-target trees) and non-target microorganisms.

4.2.2 Effects on Aquatic Organisms

A request to waive the requirement for toxicity testing on non-target aquatic organisms (including freshwater fish, aquatic arthropods, and aquatic plants) was accepted based on the following rationale. The MPCA in Rotstop C, *P. gigantea* strain VRA 1992, is a naturally occurring wood-decay fungus which was isolated from a red pine stump in Quebec, and is therefore a natural component of Canadian forest ecosystems. Any *P. gigantea* spores that may indirectly reach aquatic environments are expected to behave as they would in nature and therefore are not expected to proliferate in aquatic environments.

The potential for drift or run-off to aquatic environments during application is also negligible as the product is applied as a coarse spray (or with a brush) in a thin layer directly onto the stump surface. As it is quickly absorbed into the stump there is no direct exposure to aquatic environments and one application provides protection for several years. Furthermore, compared to basidiospores, oidia of *P. gigantea* are not long-lived in the forest environment. Consequently, the increased exposure to aquatic species from the use of Rotstop C in forests is expected to be negligible.

The extensive literature search revealed no reports of toxicity or adverse effects from *P. gigantea* to fish or other aquatic organisms, and forestry products containing *P. gigantea* have been widely used in Europe for decades with no reports of adverse effects to aquatic systems. Natural populations of the MPCA have not been associated with any adverse effects in freshwater or estuarine/marine fish species and the MPCA does not appear on any authoritative list of freshwater or estuarine/marine fish pathogens.

Based on all the available data and information on the effects of *P. gigantea* strain VRA 1992 to aquatic organisms, there is reasonable certainty that the proposed use of Rotstop C in forestry will not cause harm to non-target aquatic organisms. As a general precaution, label statements will be added to the label requiring handlers to not contaminate irrigation or drinking water or aquatic habitats by cleaning of equipment or disposal of wastes.

4.3 Incident Reports related to the Environment

Since 26 April 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website www.healthcanada.gc.ca/pesticideincident. Only incidents in which the pesticide is determined to be linked to the effects (Canadian causality of highly probable, probable and possible; U.S. causality of highly probable, probable and possible) are considered in the reviews.

As there are currently no products containing *P. gigantea* registered in Canada and the United States, there were no environmental incidents reported in the PMRA Incident reporting database nor in the USEPA's Ecological Incident Information System (EIIS) as of 21 June 2013.

5.0 Value

5.1 Effectiveness Against Pests

5.1.1 Acceptable Efficacy Claim: Control of Root and Butt Rot on Susceptible Conifer Species

A large data package including laboratory, greenhouse, field and simulated field studies from Europe and Canada was provided in support of Rotstop C's registration. European efficacy trials tested different Rotstop formulations containing local strains of *P. gigantea*. The physical, chemical and technical properties of the different Rotstop formulations are identical; only the fungal strain varies. The European data were considered adequate for review based on supportive bridging trials comparing Rotstop C with European formulations.

The various Rotstop formulations, including Rotstop C, consistently controlled the incidence and severity of root and butt rot when applied at 1 g/L water/m^2 on several conifer species. Disease reduction was well above 80% in the majority of trials. The efficacy and/or colonizing ability of *P. gigantea* strains was demonstrated on douglas-fir, hybrid larch, black pine, Italian stone pine, red pine, Scots pine, white pine, Norway spruce and Sitka spruce. Product efficacy was similar whether applied manually or with mechanized equipment. Based on this value information, the use of Rotstop C is supported for control of root and butt rot on susceptible conifer species.

5.2 Economics

Estimating the economic benefits of a treatment constitutes a challenge in forestry due to the long periods between investment and profit. Moreover, *H. irregulare* causes primary (for example, wood decay) and secondary losses (for example, reduced tree growth) and its growth rate is likely to vary over the years, making it difficult to accurately determine the cost associated with root and butt rot. For example, the cost/benefit ratio resulting from treatment with *P. gigantea* in Poland pine stands was estimated between 1:23 and 1:47 over ten years, depending on disease pressure and the type of thinning adopted. On the other end, epidemiological studies performed under British and Finnish conditions showed that treatment with a European Rotstop formulation on spruce was economically beneficial only when infection levels were expected to be moderate or high.

5.3 Sustainability

5.3.1 Survey of Alternatives

No fungicides are currently registered for this use in Canada.

5.3.2 Compatibility with Current Management Practices Including Integrated Pest Management

Rotstop C is to be applied manually or using mechanized equipment. The use of Rotstop C may be readily integrated in harvesting operations.

5.3.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

Several genes would have to be mutated for *Heterobasidion irregulare* to outcompete the biocontrol agent *P. gigantea*. The next generation would need to inherit the mutated genes while retaining pathogenicity and fitness. *Heterobasidion* species and *P. gigantea* have co-existed for centuries in nature and no cases of resistance have been reported in two decades of Rotstop use in Europe. Based on these considerations, resistance development in *Heterobasidion* species is not considered a major concern.

5.3.4 Contribution to Risk Reduction and Sustainability

H. irregulare is particularly damaging to managed red pine plantations where thinning and harvesting operations occur. Consequently, it has the potential to significantly impact the Canadian forest industry, as large plantation areas are dedicated to this coniferous species in Eastern Canada. The area of red pine plantations of thinning age (2000-2020) is estimated at 62,000 ha in Quebec and 36,000 ha in Ontario. Moreover, jack pine is also susceptible to this pathogen and *H. irregulare* was found less than 50 km from natural stands of jack pine in Quebec. In the last 10 years, an area of 131,000 ha has been planted to jack pine in Quebec. Applying the microbial fungicide Rotstop C represents a low-risk approach that contributes to forest sustainability by preventing the spread of root and butt rot, which is still restricted to specific areas.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

The Toxic Substances Management Policy (TSMP) is a federal government policy developed to provide direction on the management of substances of concern that are released into the environment. The TSMP calls for the virtual elimination of Track 1 substances [those that meet all four criteria outlined in the policy: in other words, persistent (in air, soil, water and/or sediment), bio-accumulative, primarily a result of human activity and toxic as defined by the *Canadian Environmental Protection Act*].

The technical product, Phlebiopsis gigantea strain VRA 1992, and Rotstop C were assessed in accordance with the PMRA Regulatory Directive DIR99-03.[5]

- Phlebiopsis gigantea strain VRA 1992 does not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.
- There are also no formulants, contaminants or impurities present in the end-use product that would meet the TSMP Track-1 criteria.

6.2 Formulants and Contaminants of Health or Environmental Concern

During the review process, contaminants in the technical and formulants and contaminants in the end-use products are compared against the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* maintained in the *Canada Gazette*[6]. The list is used as described in the PMRA Notice of Intent NOI2005-01[7] and is based on existing policies and regulations including DIR99-03 and DIR2006-02,[8] and taking into consideration the Ozone-depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

- The technical, Phlebiopsis gigantea strain VRA 1992, does not contain formulants of health or environmental concern as identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641-2643: *List of Pest Control Product Formulants of Health or Environmental Concern*.

- The end-use product, Rotstop C, does not contain formulants of health or environmental concern as identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641-2643: *List of Pest Control Product Formulants of Health or Environmental Concern*.

The use of formulants in registered pest control products is assessed on an ongoing basis through PMRA formulant initiatives and DIR2006-02.

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5 Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*.

6 *Canada Gazette*, Part II, Volume 139, Number 24, SI/2005-11-30) pages 2641-2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* and in the order amending this list in the *Canada Gazette*, Part II, Volume 142, Number 13, SI/2008-67 (2008-06-25) pages 1611-1613: *Part 1 Formulants of Health or Environmental Concern, Part 2 Formulants of Health or Environmental Concern that are Allergens Known to Cause Anaphylactic-Type Reactions and Part 3 Contaminants of Health or Environmental Concern*.

7 Notice of Intent NOI2005-01, *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern under the New Pest Control Products Act*.

8 Regulatory Directive DIR2006-02, *Formulants Policy and Implementation Guidance Document*.

7.0 Summary

7.1 Methods for Analysis of the Microorganism as Manufactured

The product characterization data for *Phlebiopsis gigantea* strain VRA 1992 and Rotstop C were deemed adequate to assess their potential human health and environmental risks. The technical grade of the active ingredient was characterized and the specifications of the EPs were supported by the analyses of a sufficient number of batches. Storage stability data were sufficient to support a shelf life of one year when refrigerated below 8°C or five months at room temperature (22°C).

7.2 Human Health and Safety

The acute toxicity and infectivity studies and other relevant information submitted in support of *P. gigantea* strain VRA 1992 were determined to be sufficiently complete to permit a decision on registration. Submitted information suggests *P. gigantea* strain VRA 1992 is of low toxicity by the oral, pulmonary, intraperitoneal and dermal routes, and is not pathogenic or infective via the oral, pulmonary and intraperitoneal injection exposure routes in animals. The technical grade active ingredient and the end-use product are considered to be potential sensitizers.

When handled according to prescribed label instructions, the potential for dermal, eye and inhalation exposure for mixer/loaders, and handlers and some applicators exists, with the primary source of exposure to workers being dermal and to a lesser extent inhalation.

Label statements (in other words, Potential Sensitizer, may cause sensitization, avoid breathing dust and sprays and avoid contact with eyes skin and clothing) and risk mitigation measures, such as personal protective equipment, including waterproof gloves, long-sleeved shirts, long pants, a dust-mist filtering respirator/mask (NIOSH approval number prefix TC-21C) or NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter for biological products), and shoes plus socks are required to minimize exposure and protect applicators, mixer/loaders, and handlers that are likely to be primarily exposed.

The health risk to the general population, including infants and children, as a result of bystander exposure and/or chronic dietary exposure is expected to be minimal.

7.3 Environmental Risk

The scientific rationales and supporting published scientific literature and the non-target organism study on honeybees submitted in support of Phlebiopsis gigantea strain VRA 1992 and Rotstop C (containing *P. gigantea* strain VRA 1992) were determined to be sufficiently complete. The use of Rotstop C, containing *P. gigantea* strain VRA 1992, is not expected to pose a risk to birds, mammals, arthropods, fish, and plants when the directions for use on the label are followed. No other environmental fate studies or non-target organism studies are required to assess the risk of Rotstop C used as a commercial-class biological fungicide to control root rot disease in forestry.

As a specific precaution, the Rotstop C label instructs users to not contaminate irrigation or drinking water supplies or aquatic habitats by application of product, cleaning of equipment or disposal of wastes.

7.4 Value

The value information data submitted to register Rotstop C is adequate to support the claim of control of root and butt rot on susceptible conifer species.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of Phlebiopsis gigantea strain VRA 1992 and Rotstop C, containing the technical grade active ingredient *Phlebiopsis gigantea* strain VRA 1992, to control root and butt rot, caused by *Heterobasidion irregulare*, on susceptible conifer species.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

List of Abbreviations

µg	microgram(s)
bw	body weight
CFU	colony forming unit
DACO	data code
DNA	deoxyribonucleic acid
EIIS	USEPA's Ecological Incident Information System
EP	end-use product
g	gram(s)
h	hour (s)
ha	hectare(s)
kg	kilogram(s)
km	kilometre(s)
L	litre(s)
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LOC	Level of concern
m^2	metre(s) squared
MAS	maximum average score
MCC	maximum challenge concentration
mg	milligram(s)
MIS	maximum irritation score
mL	millilitre(s)
mm	millimetre(s)
MPCA	microbial pest control agent
MPN	most probable number
MRL	maximum residue limit
NIOSH	National Institute of Occupational Safety and Health
PMRA	Pest Management Regulatory Agency
RQ	Risk Quotient
SEM	scanning electron microscopy
TEM	transmission electron microscopy
TSMP	Toxic Substances Management Policy
USEPA	United States Environmental Protection Agency
w/w	weight per weight dilution
w/v	weight per volume dilution

Appendix I Tables and Figures

Table 1.0 Toxicity and Infectivity of *P. gigantea* and its associated end-use product (Rotstop C)

Study Type	Species, Strain, and Doses	Results	Comments	Reference(s)
Acute Toxicity/Infectivity of Rotstop (Finnish strain VRA 1835)				
Acute Oral Infectivity and Toxicity (22-Day study)	Rat- Sprague-Dawley 6/sex 4.26×10^7 CFU/kg bw Interim sacrifices on Days 2, 4, 8, 15, 22 (22-day study) Untreated control group: 3/sex Shelf control group: 5/sex Autoclaved test substance: 3/sex	$LD_{50} > 4.26 \times 10^7$ CFU/kg bw	There was a rapid loss of viability following gavage. There were no deaths in any of the treatment groups and no evidence of treatment related body weight changes. No viable *P. gigantea* strain VRA 1835 was recovered from any organ, blood, intestinal contents or fecal sample from treated rats. **LOW TOXICITY NO PATHOGENICITY NO INFECTIVITY ACCEPTABLE**	PMRA 2237851
Pulmonary Infectivity and Toxicity (Intratracheal)	Rat- Sprague-Dawley 18/sex 1.12×10^6 CFU/kg bw Interim sacrifices on Days 1, 2, 4, 8, 15, 22 (21-day study) Autoclaved test substance: 3/sex 5/sex untreated control 3/sex shelf-control	$LD_{50} > 1.12 \times 10^6$ CFU/kg bw	Within 24 hours of dosing 5 rats died in groups treated with viable and autocalved test substance. Also soon after dosing in these groups there was a number of clinical signs (including but not limited to, lethargy, piloerection, decreased and increased respiratory rate unsteadiness and red staining) that were resolved by Day 1. The deaths and clinical signs were attributed to the effects of the anesthesia and trauma of the dosing procedure. There was rapid loss of viability of the test organism following intratracheal dosing. The test substance was only recovered from the lungs	PMRA 2237852

Study Type	Species, Strain, and Doses	Results	Comments	Reference(s)
			of those animals that died on Day 1. There was no evidence of treatment related bodyweight changes. **SLIGHT TOXICITY NO PATHOGENICITY NO INFECTIVITY ACCEPTABLE**	
Intraperitoneal Infectivity	Rat- Sprague-Dawley 21/sex $9.31 \times 10^4 - 1.27 \times 10^5$ CFU per animal Interim sacrifices on Days 4, 8, 15, 22 (22-day study) Autoclaved test substance: 3/sex 3/sex untreated control 3/sex shelf-control	$LD_{50} > 9.31 \times 10^4 - 1.27 \times 10^5$ CFU per animal	Based on the presence of white nodules on the organs of animals in three of the groups treated with the test substance, there was slight toxicity from intraperitoneal administration of Rotstop containing *P. gigantea* strain VRA 1835. These nodules were not present in any animals in the autoclaved or untreated control groups. No other abnormalities that were considered attributable to treatment with the viable organism were observed in any of the animals upon necropsy. **SLIGHT TOXICITY NO PATHOGENICITY NO INFECTIVITY ACCEPTABLE**	PMRA 2237853
Acute Dermal Toxicity	Rabbit- New Zealand white 5/sex undiluted 2000 mg/kg bw to an area of approximately 10% of total body surface, exposed for 24 hours (15-day study)	$LD_{50} > 2000$ mg/kg bw MIS of 0.3/4 at 72h	All animals were lethargic following dosing but recovered completely by Day 2. Dermal irritation was observed in 4 females and was completely resolved by Day 10. No dermal irritation or other clinical signs was observed in the remaining six animals during the study. **LOW TOXICITY ACCEPTABLE**	PMRA 2238015

Acute Irritation/Sensitization of Rotstop strain VRA 1835 (Finnish strain VRA 1835)				
Study Type	**Species, Strain, and Doses**	**Results**	**Comments**	**Reference(s)**
Eye Irritation	Rabbit-New Zealand albino, (3 ♀), 0.4 g of Rotstop (equivalent to 4.20×10^5 CFU of *P. gigantea* per gram), in sterile saline into the conjunctival sac of one eye, instilled for entire study. Observed for 14 days after instillation	MAS= 8.5 (2 day time point)	Corneal opacification and conjunctival irritation seen. However, because the Rotstop formulation contained a red dye the results of the conjunctival irritation were inconclusive. The reactions had resolved by Day 7 or Day 14. **NON TO MINIMALLY IRRITATING ACCEPTABLE**	PMRA 2238018
Dermal sensitization	Rabbit- New Zealand White, 20 young adults, 0.5 mL (equivalent to1.07 $\times 10^7$ CFU *P. gigantea* strain VRA 1835/g), in sterile physiological saline) Induction phase: Days 1, 8 and 15. Induction sites were evaluated 24 hours after removal then daily for 15 days. Challenge phase: 2 weeks after the last induction. Challenge sites were evaluated 24 and 48 hours after removal of test substance.	Not a dermal sensitizer	**ACCEPTABLE**	PMRA 2237850

MIS=Maximum irritation score
MAS=Maximum average score

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Test Substance	Endpoint Value	Significant Effects and Comments	Reference(s)[1]
Terrestrial Organisms					
Vertebrates					
Birds	Oral Pulmonary Injection	No study was submitted. The waiver request was based on the fact that the MPCA is a naturally occurring wood decay fungus isolated from a red pine stump in Quebec, and that the use pattern which is not expected to considerably increase the			2237922[1] 2237706

Organism	Exposure	Test Substance	Endpoint Value	Significant Effects and Comments	Reference(s)[1]
		natural background levels of the MPCA; the toxicity profile of the MPCA from the Tier I acute toxicity studies; the avian body temperature which is not conducive to growth of *P. gigantea* strain VRA 1992; the literature reported no adverse effects in wild bird populations; and the history of use of the MPCA in Europe with no reports of adverse effects to non-target terrestrial animals, including birds. WAIVER ACCEPTED			
Wild mammals	Acute	No study was submitted. The waiver request was based on the fact that the MPCA is a naturally occurring wood decay fungus isolated from a red pine stump in Quebec, and that the use pattern which is not expected to considerably increase the natural background levels of the MPCA; the toxicity profile of the MPCA from the Tier I acute toxicity studies; the literature reported no adverse effects in wild mammal populations; and the history of use of the MPCA in Europe with no reports of adverse effects to non-target terrestrial animals, including mammals. WAIVER ACCEPTED			2237922 2237706
Invertebrates					
Honeybees (*Apis mellifera*)	Acute - Contact or Oral Toxicity Laboratory study with Rotstop[2] (4×10^6 CFU/g)	**Contact exposure in 0.1% wetting agent[3]:** 0.01µg/bee 0.1 µg/bee 1.0 µg/bee 10 µg/bee 100 µg/bee **Oral exposure in 50% sucrose solution[3]:** 0.01µg/bee 0.1 µg/bee 1.0 µg/bee 10 µg/bee 100 µg/bee **Limit test:** 100 µg/bee by contact or in the diet. Observed for 48 hours	Examined at 4, 24 and 48 hours. Vehicle control group (wetting agent) Untreated control group Toxic reference study (dimethoate)	Maximum mortality in all treatment groups was 15.8%; Mortality in untreated control groups ≤10% LC_{50} > 100 µg/bee ACCEPTABLE	2238020

Organism	Exposure	Test Substance	Endpoint Value	Significant Effects and Comments	Reference(s)[1]
Terrestrial arthropods	A review of published literature was submitted in lieu of testing. Scientific reports describe an association between the red turpentine beetle (*Dendroctonus valens*) and *P. gigantea;* the fungus was found growing within the insect galleries and was readily isolated from live beetles. In contrast, negative effects on the large pine weevil from *P. gigantea* have been reported from laboratory and field studies. Pine branches inoculated with PgIBL[4] in vitro were unacceptable as oviposition substrates for adult weevils and were not conducive to larval development. In field trials, treated stumps inhibited colonization by the weevil up to a year after treatment, as the fungus consumed the nutrients which would otherwise be available to the insects. The fewest number of live larvae and the highest number of dead larvae were also found in root fragments of treatment stumps. ACCEPTED				2237922 2237706 2238021 2238022 2237023
Non-arthopod invertebrates (for example, earthworms)	No study was submitted. The waiver request was based on the fact that the MPCA is a naturally occurring wood decay fungus isolated from a red pine stump in Quebec, and that the use pattern which is not expected to considerably increase the natural background levels of the MPCA; the literature reported no adverse effects in earthworms or other non-arthropod invertebrates; and the history of use of the MPCA in Europe with no reports of adverse effects to non-arthropod invertebrates. WAIVER ACCEPTED				2237922 2237706
Soil micro-organsisms	No study was submitted. The waiver request was based on the fact that the MPCA is a naturally occurring wood decay fungus isolated from a red pine stump in Quebec and natural background levels are not expected to be considerably increased in the soil from the proposed use, and therefore environmentally or economically important microbial species are not expected to be affected, nor are any microbiologically-mediated biogeochemical processes expected to be affected; and the history of use of the MPCA in Europe with no reports of adverse effects to soil-microorganisms. WAIVER ACCEPTED				2237922
Other: Stump mycoflora	A review of published literature was submitted in lieu of testing. Scientific reports describe various Canadian and European field studies with the MPCA which conclude that effects on fungal species composition can be expected as the MPCA outcompetes certain fungi for substrate and living space. Fungal populations are expected to gradually re-establish as the natural fungal processes take place in the treated stump. Furthermore, forestry products containing *P. gigantea* have been used in Europe for decades with no evidence of an increase in incidence of fungal disease in forestry. Also as the MPCA is native to Canadian forests, it is not expected that artificial introduction of a native species, particularly from a single application at such low levels, would greatly alter the natural fungal population. ACCEPTABLE				2237922 2237706 2237763 2237858 2238036 2237861 2237859
Vascular Plants					
Terrestrial plants – ground vegetation	A review of a published study was submitted in lieu of testing. In the study Norway spruce stumps were treated with *P. gigantea* (1 g/m^2) and ground vegetation (bryophytes and vascular plant species) near the stumps were monitored for phytotoxic effects. Compared to urea and borate treatments				2237922 2237706 2238030

Organism	Exposure	Test Substance	Endpoint Value	Significant Effects and Comments	Reference(s)[1]
	which showed significant deleterious effects even after a year, no discernible impact on the ground vegetation was observed from *Phlebiopsis gigantea* treatments. ACCEPTABLE				
Terrestrial plants - Non-target trees	A review of a published study was submitted in lieu of testing. Young, non-suberized Norway spruce seedling roots were inoculated with *P. gigantea* in vitro and examined for colonisation using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). *Phlebiopsis gigantea* did not invade/disrupt the vascular systems of the root and tissue colonisation was primarily restricted to intercellular hyphal development within the middle lamella. Although cellular features necessary for pathogenicity were observed, *P. gigantea* should be considered a saprotroph. *Phlebiopsis gigantea* is not well adapted to colonizing tree roots. ACCEPTABLE				2237922 2237706 2238025
Terrestrial plants – Non-target trees	A review of a published study was submitted in lieu of testing. A Danish field study assessed the ability of *P. gigantea* to degrade living 43-year old Sitka spruce (*Picea sitchensis*). Dowels inoculated with Rotstop[2] were inserted into a drill hole that had been made into trees. Samples collected from the sapwood over a year later were examined for degradation using scanning electron microscopy (SEM). The fungus penetrated the sapwood causing some decay through degradation of cellulose and lignin, demonstrating that high inoculum concentrations (unspecified levels) are able to penetrate living Sitka spruce sapwood. The observed infectivity arose following artificial conditions (in other words, by-passing protective barrier, direct inoculation at high levels). ACCEPTABLE				2237922 2237706 2237026
Terrestrial plants - Non-target trees	A review of published literature was submitted in lieu of testing. Artificially created wounds in mature Norway spruce trees were inoculated with *P. gigantea* and other left open to natural colonisation. After a year, *P. gigantea* was only re-isolated from only half of the inoculated wounds, as well as from an additional two wounds which had not been artificially inoculated with the fungus (in other words, control trees). Infection by *P. gigantea* was favoured in wounds of suppressed trees and those which extended deeper to the heartwood. Three years after treatment, even the wounds previously inoculated with the MPCA were colonized more aggressively by fungi other than *P. gigantea* (for example, *Sternum sanguinolentum*). The infectivity potential of natural populations of *P. gigantea* has also been investigated by wounding mature Norway spruce trees. During the 4-year study, colonization of the wounds by natural populations of *P. gigantea* was very low, and colonization only occurred in deep wounds; shallow wounds of living trees do not provide a suitable environment for *P. gigantea* under natural settings. Compared with the other fungal species (*Nectria fuckeliana, Sternum samuinolentum, Cylinderobasidium evolvans*, etc.), the level of colonisation of wounds in live trees by *P. gigantea* in nature is extremely low. ACCEPTABLE				2237922 2237706 2238028 2238027 2238029

Organism	Exposure	Test Substance	Endpoint Value	Significant Effects and Comments	Reference(s)[1]
Aquatic Organisms					
Aquatic Vertebrates/Invertebrates/Plants					
Freshwater fish Arthropods and Plants	No study was submitted. The waiver request was based on the fact that the MPCA is a naturally occurring wood decay fungus isolated from a red pine stump in Quebec; that exposure is to aquatic environments is expected to be negligible; and any spores of *P. gigantea* strain VRA that may indirectly reach aquatic environments are expected to behave as they would in nature; a literature search which reported no adverse effects to aquatic organisms; and the history of use of the MPCA in Europe with no reports of adverse effects to aquatic organisms. WAIVER ACCEPTED				2237922

[1] The waiver requests for birds, wild mammals, non-arthropod invertebrates, soil microorganisms and aquatic organisms were discussed together in two reports (one for technical grade active ingredient, one for end-use product). The same documents included the literature reviews submitted in support of non-target trees/plants, mycoflora, and terrestrial arthropods. References for individual published studies (also submitted by the applicant) are also listed as appropriate.

[2] Rotstop: European formulation containing 4×10^6 CFU *P. gigantea*/g.

[3] equivalent to 0.04, 0.4, 4.0, 40, and 400 CFU/bee

[4] PgBIL is a registered biocontrol product containing a Polish isolate of *P. gigantea*

Table 3 Use (Label) Claims Proposed by Applicant and Whether Acceptable or Unsupported

Proposed claim	Supported / Unsupported
Conifers: control of root and butt rot (*Heterobasidion irregulare*) with one preventative stump treatment at a minimum of 1g Rotstop C / L water / m^2 of stump area.	Supported on susceptible conifer species, as proposed.

References

A. List of Studies/Information Submitted by Registrant

1.0 Chemistry

PMRA	Reference
2237714	1978, The Corticaceae of North Europe, Vol. 5, DACO: IIM 1.3.1, M2.7.1
2237715	1981, The Corticaceae of North Europe, Vol. 6, DACO: IIM 1.3.1, M2.7.1
2237716	1988, The sexuality of *Phlebiopsis gigantea*, DACO: IIM 1.3.1,M2.7.1
2237717	1987, Genetic variation in *Phlebiopsis gigantea* as detected with random amplified microsatellite (RAMS) markers, DACO: IIM 1.3.1,M2.7.1
2237718	2000, Genetic differentiation between European and North American populations of *Phlebiopsis gigantea*, DACO: IIM 1.3.1,M2.7.1
2237719	2000, A taxonomic study of phleboid fungi (Basidiomycota), DACO: IIM 1.3.1,M2.7.1
2237720	2003, Phylogenetic relationships of the genus Phanerochaete inferred from the interna transcribed spacer region, DACO: IIM 1.3.1,M2.7.1
2237721	2003, New isolates of *Phlebiopsis gigantea*; methods and results., DACO: IIM 1.3.1,M2.7.1
2237722	2005, Potential for biological control of *Heterobasidion annosum* in the UK using Rotstop, DACO: IIM 1.3.1,M2.7.1
2237723	2005, Interfertility between North American and European strains of *Phlebiopsis gigantea*, DACO: IIM 1.3.1,M2.7.1
2237730	1992, Identification of the fungus from the biopreparate made by Kemira Oy for conifer stump treatment, DACO: IIM 1.3.3,M2.7.1
2237731	2004, Identification of fungal culture, Certificate, DACO: IIM 1.3.3,M2.7.1
2237732	2001, Persistence of a biological strain of *Phlebiopsis gigantea* in conifer stumps and its effect on within-species genetic diversity, DACO: IIM 1.3.3,M2.7.1,M8.1,M9.7,M9.8.1
2237733	2003, Identification of fungal isolates from the biopreparates 1984, 1985 and 1986, made by Verdera Oy for treating conifer stumps against Heterobasidion, DACO: IIM 1.3.3,M2.7.1
2237734	1997, Comparison of RAPD technique and somatic incompatibility tests for the identification of *Phlebiopsis gigantea* strains, DACO: IIM 1.3.3,M2.7.1,M9.7
2237746	2000, Comparison of three products based on *Phlebiopsis gigantea* for the control of *Heterobasidion annosum* in Europe, DACO: IIM 1.4.7,M1.3
2237747	1963, Stump protection against *Fomes annosus*. III. Inoculation with *Peniophora gigantea*, DACO: IIM 2.1,M2.7.1,M2.7.2

2237748	1973, An alternative to chemical stump protection against *Fomes annosus* on pines in state and private forestry, DACO: IIM 2.1,M2.7.1, M2.7.2
2237749	1975, Biological control of *Fomes annosus* by *Peniophora gigantea*, DACO: IIM 2.1, M2.7.1, M2.7.2
2237750	2002, Fomes root rot in Thetford Forest, East Anglia: past, present and future, DACO: IIM 2.1,M2.7.1,M2.7.2
2237751	2001, Efficiency of *Phlebiopsis gigantea* in PgIBL Preparation to control the root rot disease in threatened Scots pine stands in the last decade of 2000, DACO: IIM 2.1,M2.7.1,M2.7.2
2237752	1979, Control of *Heterobasidion annosum* (Fr) Bref (*Fomes annosus*) in Finland, DACO: IIM 2.1,M2.7.1,M2.7.2
2237753	1994, Control of *Heterobasidion annosum* by stump treatment with Rotstop - a new commercial formulation of *Phlebiopsis gigantea*, DACO: IIM 2.1,M2.7.1,M2.7.2
2237754	1997, *Heterobasidion annosum* infection following mechanized first thinning and stump treatment in *Picea abies*, DACO: IIM 2.1,M2.7.1,M2.7.2
2237756	1992, An evaluation of six methods for protecting red pine stumps from infection by *Fomes annosus* in Ontario., DACO: IIM 2.1,M2.7.1,M2.7.2
2237757	1994, Annosus root rot caused by *Heterobasidion annosum*, DACO: IIM 2.1,M2.7.1,M2.7.2
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2237759	1996, Lutte contre la maladie du rond dans l'ouest du Quebec (Annosus root rot control in Western Quebec), DACO: IIM 2.1, M2.7.1, M2.7.2
2237762	2003, Field tests on biological control of *Heterobasidion annosum* by *Phaeotheca dimorphospora* in comparison with *Phlebiopsis gigantea*, DACO: IIM 2.1,M2.7.1,M2.7.2
2237765	1998, Biological methods of control, DACO: IIM 2.2,M2.7.2
2237766	1998, Registration of *Phlebiopsis gigantea* as a forest biolcontrol agent in the UK: recent experience, DACO: IIM 2.2,M2.7.2
2237768	1963, Biological control of the root-rot fungus *Fomes annosus* (Fr)Cke by *Peniophora gigantea* (Fr) Massee, DACO: IIM 2.2,M2.7.2
2237771	1957, Investigation of the fungal flora of spruce and pine stumps, DACO: IIM 2.2,M2.7.2,M9.7
2237775	1970, Aerial distribution of the root-rot fungus *Fomes annosus* (Fr) Cooke in Finland, DACO: IIM 2.2,M2.7.2
2237777	1977, Microbial flora isolated from Norway spruce stumps (Kuusen kantojen mikrobi-lajisto), DACO: IIM 2.2,M2.7.2
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2237829	2008, Viability determination of biological pesticides using MPN method, DACO: IIM 4.3.1,M2.10.1
2237830	1992, Isolation of *P. gigantea* from tree stump or log, DACO: IIM 4.3.1,M2.10.1
2237831	2005, Rotstop test on agar plates, DACO: IIM 4.3.3,M2.10.1
2237832	2003, Simulated stump treatment experiments for monitoring the efficacy of *Phlebiopsis gigantea* against Heterobasidion, DACO: IIM 4.3.3,M2.10.1
2237833	2005, Quality control in Rotstop production facilities, DACO: IIM 4.3.5,M2.10.3,M2.8,M2.9.3
2237834	2005, Method for surveying viable microbes in the indoor air in the production facilities of Rotstop, DACO: IIM 4.3.5,M2.10.3,M2.8,M2.9.3
2237835	2005, Storage stability of biological control agents, DACO: IIM 4.4,M2.11
2237862	2008 SANCO 1863/08 - rev. 3, , Review report on the active substance *Phlebiopsis gigantea*, DACO: 12.5 (OECD)
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2237937	2005, Analysis of pathogenic contaminants in Rotstop, DACO: IIIM 1.7.3.2,M2.10.2,M2.8,M2.9.3 CBI
2237938	2005, Five batch analysis of Rotstop, DACO: IIIM 1.7.4,M2.10.2,M2.11,M2.8,M2.9.1 CBI

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4.0 Value

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B. Additional Information Considered

i) Published Information

2336567 European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance *Phlebiopsis gigantea*. EFSA Journal 2013;11(1):3033. [31 pp.] doi:10.2903/j.efsa.2013.3033. Available online: www.efsa.europa.eu/efsajournal